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PUBLICATION
S. Lee, M.E. Sowa, Y. Watanabe, P.B. Sigler, W. Chiu, M. Yoshida and F.T.F. Tsai., "The Structure of ClpB: A Molecular Chaperone that Rescues Proteins from an Aggregated State," *Cell,* **115**, 229-240 (2003a).

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Structure of the ClpB Molecular Chaperone

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Molecular chaperones are cellular proteins that assist other proteins in the folding and assembly of higher order structures without being components of these final structures. ClpB is an essential protein of the bacterial heat-shock response, but unlike other chaperones, has the remarkable ability to rescue proteins from an aggregated state. The 3.0 angstrom (Å) resolution crystal structure of ClpB reveals that the ClpB middle region forms an 85 Å-long, mobile coiled-coil structure, which resembles the shape of a two-bladed propeller. The conformation and motion of this coiled-coil are critical for ClpB's chaperone function. The coiled-coil is located on the outside of the ClpB hexamer, where it can interact with aggregated proteins. This suggests that the long coiled-coil may function as a "molecular crowbar" that mediates protein disaggregation.

Bacterial ClpB is a molecular chaperone that requires adenosine triphosphate (ATP) for function. Members of the ClpB family form large ring structures and contain two ATP-binding domains. Moreover, ClpB has a longer middle region, known as the ClpB-linker, and, unlike other Clp proteins, does not direct the degradation of its substrate proteins. Instead, ClpB has the remarkable ability to rescue stress-damaged proteins from an aggregated state.

Despite the wealth of biochemical and genetic data, the mechanism by which ClpB disaggregates stress-damaged proteins has remained elusive. To investigate this structure-function relationship, we have determined the 3.0 Å-resolution crystal structure of ClpB bound to adenosine 5'-(β,γ-imido)triphosphate (AMPPNP), an ATP analog. We have also determined the structure of the physiological assembly at approximately 21 Å resolution using electron cryomicroscopy (cryo-EM) (Lee *et al.*, 2003a; 2003b). Our crystal structure reveals that ClpB consists of five domains (**Figure 1A**). The most remarkable structural feature is its 85 Å-long coiled-coil that consists of the ClpB-linker. There are three independent representations of ClpB in the asymmetric unit of our crystal. The three molecules are arranged in a manner that gives rise to a helical assembly (**Figure 2A**), which extends throughout the crystal and forms a hexameric ring structure when viewed in projection (**Figure 2B**). Interestingly, each ClpB molecule adopts a different conformation even though they are in the same nucleotide-bound state. This suggests that ClpB is a dynamic molecule that can undergo large conformational rearrangements. Using a combination of structure-based targeted mutagenesis and biochemical experiments, we have further demonstrated that the conformation and motion of the long coiled-coil are critical for chaperone activity (Lee *et al.*, 2003a).

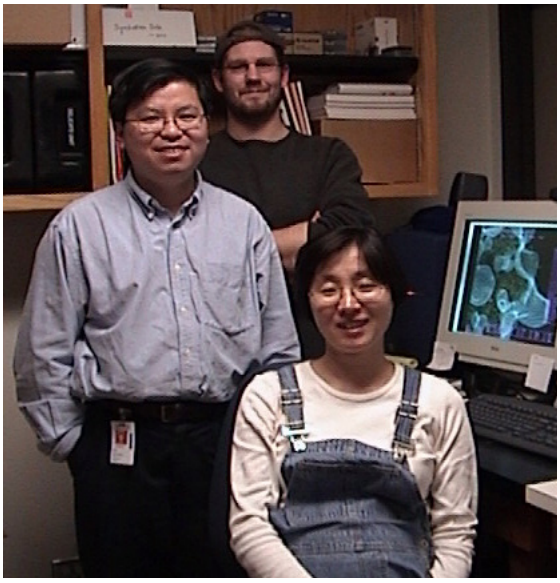

Photo of the Research Group: Authors (from left): Francis T.F. Tsai, Mathew E. Sowa and Sukyeong Lee.

The structure of the functional ring assembly was determined using cryo-EM and single-particle reconstruction techniques of ClpB-AMPPNP complexes (Lee *et al.*, 2003a). Our three-dimensional reconstruction shows that ClpB is a hexamer and that both of its ATP-binding domains are required for hexamer formation in the AMPPNP-bound state (**Figure 1B**). An atomic model of the ClpB hexamer was generated by fitting the crystal structure of ClpB into the cryo-EM density map (**Figure 1B**). Our model reveals that the ClpB-linker is located on the outside of the hexamer, where it can interact with large aggregated substrate proteins. Taken together, our

structural and biochemical studies support a mechanism in which the long coiled-coils function as "molecular crowbars" that pull apart large aggregates, thereby mediating protein disaggregation.

Additional Publication:
S. Lee, M. Hisayoshi, M. Yoshida, and F.T.F. Tsai, "Crystallization and Preliminary X-ray Crystallographic Analysis of the Hsp100 Chaperone ClpB from *Thermus Thermophilus*," *Acta Crystallogr., D***59**, 2334-2336 (2003b).

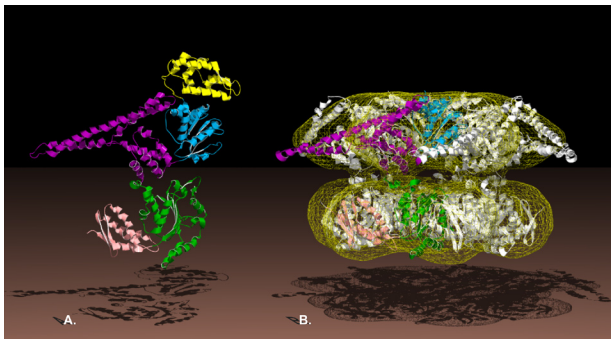

Figure 1: *(A) Ribbon drawing of the ClpB monomer. The N-terminal domain is colored in yellow, the D1-large domain or NBD1 in cyan, the D1-small domain in purple, the D2-large domain or NBD2 in green, and the D2-small domain in pink. The bound AMPPNP molecules have been omitted for clarity. (B) Single-particle reconstruction of the ClpB hexamer. The cryo-EM map is contoured as a yellow mesh, with the atomic coordinates of an N-terminal domain truncated ClpB hexamer docked in. The N-terminal domains and part of the ClpB-linker are not visible in the reconstruction because they are mobile.*

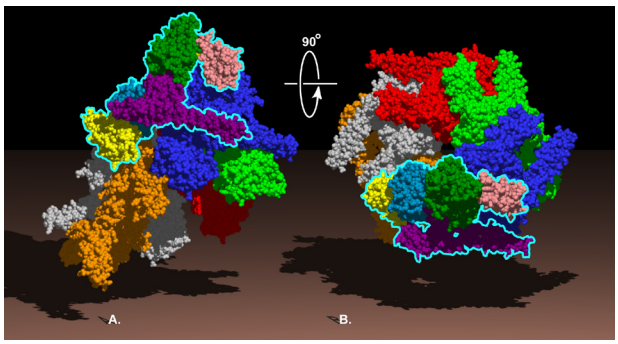

Figure 2: *Molecular arrangement of six ClpB molecules as seen in our crystal structure. The figure illustrates the molecular contents of two asymmetric units, which are related by a crystallographic two-fold screw axis. Each ClpB monomer is colored differently. To depict the relative orientation of ClpB in our crystal, one of the six molecules has been highlighted and color-coded as shown in Figure 1A.*